

Thermodynamic and Kinetic Studies on Reactions of Pt(II) Complexes with Biologically Relevant Nucleophiles

Nadine Summa,[†] Wolfgang Schiessl,[†] Ralph Puchta,^{†,‡} Nico van Eikema Hommes,[‡] and Rudi van Eldik^{*,†}

Institute for Inorganic Chemistry, University of Erlangen-Nürnberg, Egerlandstrasse 1, 91058 Erlangen, Germany, and Computer Chemistry Center, University of Erlangen-Nürnberg, Nägelsbachstrasse 25, 91052 Erlangen, Germany

Received November 11, 2005

The effect of different N–N spectator ligands on the reactivity of platinum(II) complexes was investigated by studying the water lability of [Pt(diaminocyclohexane)(H₂O)₂]²⁺ (**Pt(dach)**), [Pt(ethylenediamine)(H₂O)₂]²⁺ (**Pt(en)**), [Pt(aminomethylpyridine)(H₂O)₂]²⁺ (**Pt(amp)**), and [Pt(*N,N'*-bipyridine)(H₂O)₂]²⁺ (**Pt(bpy)**). Some of the selected N–N chelates form part of the coordination sphere of Pt(II) drugs in clinical use, as in **Pt(dach)** (oxaliplatin), or are models, regarding the nature of the amines, with higher stability in terms of substitution and hydrolysis of the diamine moiety, as in **Pt(en)** (cisplatin) and **Pt(amp)** (AMD473). The effect of π -acceptors on the reactivity was investigated by introducing one (**Pt(amp)**) or two pyridine rings (**Pt(bpy)**) in the system. The p*K*_a values for the two water molecules (viz., **Pt(dach)** (p*K*_{a1} = 6.01, p*K*_{a2} = 7.69), **Pt(en)** (p*K*_{a1} = 5.97, p*K*_{a2} = 7.47), **Pt(amp)** (p*K*_{a1} = 5.82, p*K*_{a2} = 6.83), **Pt(bpy)** (p*K*_{a1} = 4.80, p*K*_{a2} = 6.32) show a decrease in the order **Pt(dach)** > **Pt(en)** > **Pt(amp)** > **Pt(bpy)**. The substitution of both coordinated water molecules by a series of nucleophiles (viz., thiourea (tu), L-methionine (L-Met), and guanosine-5'-monophosphate (5'GMP[−])) was investigated under pseudo-first-order conditions as a function of concentration, temperature, and pressure using UV–vis spectrophotometric and stopped-flow techniques and was found to occur in two subsequent reaction steps. The following *k*₁ values for **Pt(dach)**, **Pt(en)**, **Pt(amp)**, and **Pt(bpy)** were found: tu (25 °C, M^{−1} s^{−1}) 21 ± 1, 34.0 ± 0.4, 233 ± 5, 5081 ± 275; L-Met (25 °C) 0.85 ± 0.01, 0.70 ± 0.03, 2.15 ± 0.05, 21.8 ± 0.6; 5'GMP[−] (40 °C) 5.8 ± 0.2, 3.9 ± 0.1, 12.5 ± 0.5, 24.4 ± 0.3. The results for *k*₂ for **Pt(dach)**, **Pt(en)**, **Pt(amp)**, and **Pt(bpy)** are as follows: tu (25 °C, M^{−1} s^{−1}) 11.5 ± 0.5, 10.2 ± 0.2, 38 ± 1, 1119 ± 22; L-Met (25 °C, s^{−1}) 2.5 ± 0.1, 2.0 ± 0.2, 1.2 ± 0.3, 290 ± 4; 5'GMP[−] (40 °C, M^{−1} s^{−1}) 0.21 ± 0.02, 0.38 ± 0.02, 0.97 ± 0.02, 24 ± 1. The activation parameters for all reactions suggest an associative substitution mechanism. The p*K*_a values and substitution rates of the complexes studied can be tuned through the nature of the N–N chelate, which is important in the development of new active compounds for cancer therapy.

Introduction

The antitumor activity of [*cis*-Pt(NH₃)₂Cl₂], cisplatin, opened new perspectives for other types of chemotherapeutics. It is still one of the most widely used drugs in the world, although its high activity is accompanied with severe side effects¹ and the development of resistance.^{2–4} To overcome these drawbacks, significant research efforts have focused

on designing new drugs as well as understanding their ligand exchange and DNA-platination⁵ reactions. As simple as these compounds, [PtA₂X₂] (X = anionic leaving group, A = ammonia, amine, pyridine), may look at first sight, the reactions involved in their action as a drug in a human organism are very complex. These reactions include aquation,

* To whom correspondence should be addressed. E-mail: vaneldik@chemie.uni-erlangen.de.

[†] Institute for Inorganic Chemistry.

[‡] Computer Chemistry Center.

(1) Lippert, B. e. *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*. Wiley-VCH: Zürich, Switzerland, 1999.

(2) Meijer, C.; Mulder, N. H.; Timmer-Bosscha, H.; Sluiter, W. J.; Meersma, G. J.; de Vries, E. G. *Cancer Res.* **1992**, 52 (24), 6885–6889.

(3) Fox, M.; Roberts, J. J. *Cancer Metastasis Rev.* **1987**, 6 (3), 261–281.

(4) Fokkema, E.; Groen, H. J.; Helder, M. N.; de Vries, E. G.; Meijer, C. *Biochem. Pharmacol.* **2002**, 63 (11), 1989–1996.

(5) Reedijk, J. *Chem. Rev.* **1999**, 99, 2499–2510.

anation, biotransformation reactions with proteins, RNA/DNA, and smaller molecules, for example, amino acids and inorganic anions, and resulting degradation reactions.^{6–8}

It is known that the formation of DNA-adducts is responsible for the antitumor activity of Pt(II) drugs,^{1,6} but many of the reactions mentioned above compete with this. Sulfur-containing molecules, especially, play a crucial role in drug distribution, thermodynamic and kinetic competition with nucleobase binding, and the mechanism of metabolism.⁵ As the Pt drug is administered via injection or infusion, several S-containing nucleophiles present in blood are available to react with the Pt(II) complex.⁹ This interaction has also been associated with the severe side effects of Pt(II) cytostatics.¹⁰ Many of the reactions with S- and N-donor nucleophiles are well investigated and understood,¹ although the interplay of all these processes is still controversial. From a theoretical point of view, sulfur donors win the competition for the soft-metal Pt(II) center, and studies also indicate a kinetic preference for thioethers, for example, over N-donors.^{5,11,12} However, a sufficient amount of the Pt(II) drug still reaches and reacts with the DNA target. Most likely the conditions under which the reactions proceed are of significant importance for the chosen pathway. DFT calculations performed by Deubel¹³ indicated that the dielectric constant, ϵ , in biological microenvironments is crucial for the reactivity and selectivity of Pt(II) with biological targets and also the substituents attached to the heteroatom considered. Particularly in the cell, different values for ϵ can be found. Some compartments of the cell, for example, exhibit higher concentrations of proteins and DNA and therefore exhibit another ϵ , which can have a strong influence on the stabilization of the transition state of a reaction. Competition studies for Pt–amine complexes with S-donor ligands and nucleobases have demonstrated that a transfer from a thioether ligand to a guanine-N7 site can occur, with the result that such Pt–thioether adducts can presumably serve as a drug reservoir for platination at DNA.⁵

This strong interaction between S-donor nucleophiles and Pt(II) complexes was also investigated in terms of their chemoprotective ability. Especially against nephrotoxicity, thiocarbonyls, such as thiourea, or thioethers, such as methionine, were used to prevent or reverse the formation of Pt–S adducts in proteins.^{5,14} Thiourea is also a very convenient and often employed nucleophile used to study ligand substitution reactions in coordination chemistry

because of its good solubility, neutral character, and high nucleophilicity. In addition, thiourea is a challenging molecule¹⁵ since it combines the ligand properties of thioates¹⁶ (π -donor) and thioethers¹⁷ (σ -donor, π -acceptor). Thus, there are several motivations to study the reactions of these complexes (viz., thiourea (tu), L-methionine (L-Met), and guanosine-5'-monophosphate (5'GMP⁻)) as nucleophiles. 5'GMP⁻ was used as model for binding to a nucleobase.

It was recently reported that monofunctional Pt(II) complexes with tridentate ligands (e.g., terpyridine) show remarkable trends in reaction rates, pK_a values, and nucleophilic discrimination depending on the π -acceptor properties of the spectator ligands.¹⁸ Since a bifunctional addition to DNA seems to be critical for its cytotoxic activity, we selected a series of Pt(II) complexes with either a different combination of amines or both amines and pyridines in a bidentate fashion. [Pt(ethylenediamine)(H₂O)₂]²⁺ (**Pt(en)**) was used as a model complex for cisplatin, circumventing the facile substitution of amines by using a diamine chelate. The complex [Pt(diaminocyclohexane)(H₂O)₂]²⁺ (**Pt(dach)**) can be considered to be the hydrolysis product of oxaliplatin ((*trans*-1,2-diaminocyclohexane)oxalatoplatinum(II)), a third generation platinum drug. It was found to be active in cisplatin resistant tumors and to exhibit a distinct side effect profile as cisplatin.⁶ To continue the investigations of Hofmann et al.¹⁸ in terms of the effect of π -acceptors on the thermodynamic and kinetic properties of Pt(II) complexes, we combined half of the **Pt(en)** complex with one π -acceptor ligand, resulting in [Pt(aminomethylpyridine)(H₂O)₂]²⁺ (**Pt(amp)**). In terms of the nature and electronic properties of the amines (NH₂R, pyridine), the **Pt(amp)** complex can be considered to be a model for *cis*-[PtCl₂(NH₃)(2-picoline)] (AMD473), although the methyl group of the picoline ligand in AMD473 will have an additional steric influence on the reaction rate, most likely a deceleration. AMD473 has entered clinical trials Phase I and II and shows a profile of chemical and biological activity that differs significantly from that of cisplatin.¹⁹ Finally, in [Pt(*N,N'*-bipyridine)(H₂O)₂]²⁺ (**Pt(bpy)**) two pyridine rings were introduced to further study the influence of π -acceptors on the electronic properties of the Pt(II) center.

In this work, we investigated the reactions of the complexes **Pt(dach)**, **Pt(en)**, **Pt(amp)**, and **Pt(bpy)** with tu, L-Met, and 5'GMP⁻ using UV–vis spectrophotometric and stopped-flow techniques to study the influence of electronic effects on reactions of potential biological importance. The pK_a values were determined for the two coordinated water molecules and interpreted in terms of the ability to stabilize the hydroxo species.

- (6) Jakubec, M. A.; Galanski, M.; Keppler, B. K. *Rev. Physiol. Biochem. Pharmacol.* **2003**, *146*, 1–53.
 (7) Costa, L. A. S.; Rocha, W. R.; De Almeida, W. B.; Dos Santos, H. F. *Chem. Phys. Lett.* **2004**, *387*, 182–187.
 (8) Costa, L. A. S.; Rocha, W. R.; De Almeida, W. B.; Dos Santos, H. F. *J. Chem. Phys.* **2003**, *118*, 10584–10592.
 (9) Kozelka, J.; Legendre, F.; Reeder, F.; Chottard, J.-C. *Coord. Chem. Rev.* **1999**, *190–192*, 61–81.
 (10) Dorr, R. T. *Platinum and other Metal Coordination Compounds in Cancer Chemotherapy*. Plenum: New York, 1996; pp 131–154.
 (11) Teuben, J.-M.; Reedijk, J. *J. Biol. Inorg. Chem.* **2000**, *5*, 463–468.
 (12) Fakh, S.; Munk, V. P.; Shipman, M. A.; Murdoch, P. d. S.; Parkinson, J. A.; Sadler, P. J. *Eur. J. Inorg. Chem.* **2003**, 1206–1214.
 (13) Deubel, D. V. *J. Am. Chem. Soc.* **2004**, *126*, 5999–6004.
 (14) Burchenal, J. H.; Kalaher, K.; Dew, K.; Lokys, L.; Gale, G. *Biochimie* **1978**, *60*, 961–965.

- (15) Schiessl, W. C.; Summa, N. K.; Weber, C. F.; Gubo, S.; Dücker-Benfer, C.; Puchta, R.; van Eikema Hommes, N. J. R.; van Eldik, R. Z. *Anorg. Allg. Chem.* **2005**, *631*, 2812–2819.
 (16) Ashby, M. T. *Comments Inorg. Chem.* **1990**, *10*, 297.
 (17) Murray, S. G.; Hartley, F. R. *Chem. Rev.* **1981**, *81*, 365.
 (18) Hofmann, A.; Jaganyi, D.; Munro, O. Q.; Liehr, G.; van Eldik, R. *Inorg. Chem.* **2003**, *42*, 1688–1700.
 (19) McGowan, G.; Parsons, S.; Sadler, P. J. *Chem.—Eur. J.* **2005**, *11*, 4396–4404.

Experimental Section

Chemicals and Ligands. The ligands, ethylenediamine and 2-pyridylmethylamine, as well as the nucleophiles, L-methionine, thiourea, and guanosine-5'-monophosphate sodium salt, used in the kinetic measurements, were obtained from Acros Organics. Nucleophile stock solutions were prepared shortly before use by dissolving the chemicals. The ligand 2,2'-bipyridine and the complex *trans*-1,2-diaminocyclohexanedichloroPt(II) were obtained from Aldrich. Potassium tetrachloro-platinate (K_2PtCl_4) was purchased from Strem Chemicals; 98% CF_3SO_3D (Aldrich), CF_3SO_3H (Aldrich), and 99.9% D_2O (Deutero GmbH) are commercially available and were used as received. All other chemicals were of the highest purity commercially available and were used without further purification. Ultrapure water was used in all experiments involving aqueous solutions.

Synthesis of Complexes. Literature procedures were used for the synthesis of $[Pt(ethylenediamine)Cl_2]^{20}$ (**Pt(en)**), $[Pt(bipyridine)Cl_2]$ (**Pt(bpy)**), and $[Pt(aminomethylpyridine)Cl_2]$ (**Pt(amp)**).²¹ Aqueous solutions of the complexes in their aqua form were prepared in situ by the addition of slightly less than 2 mol equiv of $AgOTf$ ($OTf = CF_3SO_3^-$) to a solution of known amounts of the chloro complex in 0.01 M HOTf. The white precipitate ($AgCl$) that formed was filtered off using a Millipore filtration unit, and the solution was diluted with 0.01 M HOTf in a graduated flask to the concentration used in the kinetic measurements. Thus, the pH was 2.0, and the ionic strength was 0.01 M.

$[Pt(ethylenediamine)Cl_2]$. A solution of ethylenediamine (73.71 mg, 1.23 mmol) in 0.01 M HCl (15 mL) was added to a 0.01 M HCl solution (15 mL) of K_2PtCl_4 (509.2 mg, 1.23 mmol). The mixture was stirred for 12 h at 55–60 °C under reflux. After 2 h, a yellow-green solid started to precipitate. The solution was allowed to cool, and the compound formed, $[Pt(en)Cl_2]$, was filtered off, washed carefully with small amounts of water, ethanol, and diethyl ether, and dried under vacuum to give analytically pure material. Yield: 275 mg (0.894 mmol, 73%). Anal. Calcd for $C_2H_8Cl_2N_2Pt$: H, 2.47; C, 7.36; N, 8.59. Found: H, 2.29; C, 7.19; N, 8.44.

$[Pt(aminomethylpyridine)Cl_2]$. A solution of 2-aminomethylpyridine (106.5 mg, 0.535 mmol) in 0.01 M HCl (15 mL) was added to a solution (15 mL) of K_2PtCl_4 (222 mg, 0.535 mmol) in 0.01 M HCl. The mixture was stirred for 12 h at 55–60 °C. After 2 h, an olive-green solid started to precipitate. The solution was allowed to cool, and the compound formed, $[Pt(amp)Cl_2]$, was filtered off, washed carefully with small amounts of water, ethanol, and diethyl ether, and dried under vacuum to give analytically pure material. Yield: 162 mg (0.433 mmol, 81%). Anal. Calcd for $C_6H_8Cl_2N_2Pt$: H, 2.16; C, 19.26; N, 7.49. Found: H, 1.99; C, 19.40; N, 7.31.

$[Pt(bipyridine)Cl_2]$. A solution of *N,N'*-bipyridine (73.99 mg, 0.474 mmol) in 0.01 M HCl (15 mL) was added to a 0.01 M HCl solution (15 mL) of K_2PtCl_4 (196.64 mg, 0.474 mmol). The mixture was stirred for 12 h at 55–60 °C under reflux. After 2 h, a yellow solid started to precipitate. The solution was allowed to cool, and the compound formed, $[Pt(bpy)Cl_2]$, was filtered off, washed carefully with small amounts of water, ethanol, and diethyl ether, and dried under vacuum to give analytically pure material. Yield: 162 mg (0.375 mmol, 79%). Anal. Calcd for $C_{10}H_8Cl_2N_2Pt$: H, 1.91; C, 28.45; N, 6.64. Found: H, 1.46; C, 28.44; N, 6.50.

Instrumentation and Measurements. NMR Spectroscopy and Elemental Analysis. NMR spectroscopy (Bruker Avance DPX 300)

Table 1. Summary of the pK_a Values Obtained for the Deprotonation of Platinum-Bound Water and Point Groups of the Different Complexes

	Pt(dach)	Pt(en)	cisplatin-(OH) ₂	Pt(amp)	Pt(bpy)
pK_{a1}	6.01	5.97	5.8 ^a	5.37 ^b	5.82
pK_{a2}	7.69	7.47	7.6 ^a	7.21 ^b	6.83
point group	C_2	C_2	C_{2v}	C_1	C_{2v}

^a Coley, R. F.; Martin, D. S. *Inorg. Chim. Acta* **1973**, *7*, 573. ^b Berners-Price, S. J.; Frenkiel, T. A.; Frey, U.; Ranford, J. D.; Sadler, P. J. *J. Chem. Soc., Chem. Commun.* **1992**, 789.

and a Carlo Erba Elemental Analyzer 1106 were used for complex characterization and chemical analysis, respectively. All chemical shifts are referenced to TSP (trimethylsilylpropionic acid) in D_2O or TMS (tetramethylsilane) in DMSO, with high-frequency shifts recorded as positive numbers. In D_2O solutions, the pH^* was measured with an inoLab SenTix Mic pH Microelectrode.

Spectrophotometric pH Titrations. The spectrophotometric pK_a determination was done using a Hellma absorption cell for flow through measurements attached to a peristaltic pump. A large volume (100 mL) of the complex solution was used to circumvent dilution. To avoid contamination by chloride released from the pH electrode, it was necessary to take 2 mL aliquots from the solution into narrow vials for the pH measurements; the aliquots were discarded after the measurement. The pH of aqueous solutions was monitored online using a Thermo Orion Ross Ultra Combination pH glass electrode linked to a computer or a WTW Inolab level 1 pH meter with a combined glass electrode. The electrodes were calibrated using standard buffer solutions of pH 4, 7, and 10 obtained from Sigma and WTW. Spectrophotometric pH titrations of the complex solutions were performed with NaOH as base at 25 °C. The addition of solid NaOH resulted in a stepwise change in pH from 2 to approximately 3. The subsequent pH changes were obtained by the dropwise addition of a saturated, 1, or 0.1 M NaOH solution using a micropipet. The complex concentration during these trials was 1.25×10^{-4} M.

UV–Vis Kinetic Experiments. UV–vis spectra were recorded on a Varian Cary 5G or Cary 1 spectrophotometer equipped with a thermostated cell holder or on a Shimadzu UV-2101-PC spectrophotometer with a thermoelectrically controlled cell holder for the determination of pK_a values and for the study of slow reactions. Kinetic measurements on fast reactions were studied on an Applied Photophysics SX 18MV or Dionex Durrum stopped-flow instrument coupled to an online data acquisition system. Experiments at elevated pressure were performed on a laboratory-made high-pressure stopped-flow instrument for fast reactions,²² and for slow reactions, they were performed on a Shimadzu UV-2101-PC spectrophotometer equipped with a laboratory-made high-pressure cell.²³ The temperature of the instruments was controlled throughout all kinetic experiments with an accuracy of ± 0.1 °C, while the ionic strength of the solution was maintained at 0.01 M using HOTf. Except for one measurement at pH 4, all reactions were studied at pH 2.0–2.2 to guarantee the presence of the diaqua form of the complexes, with respect to their pK_a values (see data in Table 1). Thus, OH-bridged or dihydroxo Pt complexes, which play an important role under physiological conditions since they are practically inert to substitution reactions,^{41,42} can be excluded.

The spectral changes of the reactions were first recorded over the wavelength range of 190–500 nm to establish a suitable

(20) Al-Baker, S.; Dabrowiak, J. C. *Inorg. Chem.* **1987**, *26*, 613.

(21) Newkome, G. R.; Theriot, K. J.; Fronczek, F. R.; Villar, B. *Organometallics* **1989**, *8*, 2513–23.

(22) van Eldik, R.; Gaede, W.; Wieland, S.; Kraft, J.; Spitzer, M.; Palmer, D. A. *Rev. Sci. Instrum.* **1993**, *64*, 1355.

(23) Spitzer, M.; Gartig, F.; van Eldik, R. *Rev. Sci. Instrum.* **1988**, *59*, 2092.

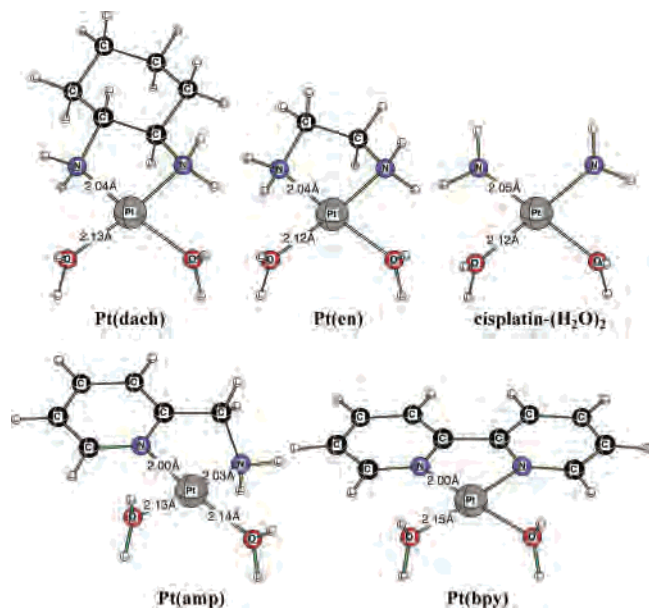


Figure 1. Calculated structures (B3LYP/LANL2DZp) of the diaqua form of cisplatin and the investigated Pt(II) complexes.

wavelength at which the kinetic trace could be followed. The wavelengths used for each reaction are listed in Table S1 (Supporting Information). The slow reactions were initiated by mixing 900 μ L of complex with 900 μ L of nucleophile solution in a tandem cuvette, thermostated in a spectrophotometer cell compartment.

The ligand substitution reactions were studied under pseudo-first-order conditions. This was achieved by using at least a 10-fold (1:1 complex formation) or 20-fold (1:2 complex formation) excess of the nucleophile or the complex. All reported rate constants represent an average value of at least three to five independent kinetic runs for each experimental condition. The temperature dependence was studied in the range of 15–40 or 25–45 °C, and the pressure dependence was studied from 1 to 130 MPa.

Results and Discussion

To investigate the impact of the different diamine chelates on the reactivity of the aqua complexes, the pK_a values of the coordinated water molecules were first determined, followed by ligand substitution reactions with the three selected nucleophiles (tu, L-Met, 5'GMP⁻). The dependence on the nucleophile concentration, temperature, and pressure resulted in rate constants and activation parameters (ΔH^\ddagger , ΔS^\ddagger , and ΔV^\ddagger) for the displacement of coordinated water.

Figure 1 shows the structures of the investigated complexes calculated with DFT (RB3LYP/LANL2DZp).^{24–32} Pt(dach) and Pt(en) both have C_2 symmetry, with a nonplanar chelate ring, whereas the planar Pt(bpy) complex has C_{2v} symmetry. The Pt(amp) complex exhibits the lowest, C_1 symmetry. The nature of the nitrogen ligands does not significantly influence the Pt–N and Pt–O bond lengths, although a slight elongation of the Pt–O bond trans to pyridine, as compared to trans to NH₂ can be recognized. No crystal structures for this type of complex, [Pt(N–N)(H₂O)₂], were found in the literature for comparison. However, the slight elongation of the Pt–O bond was also seen in the work of Hofmann et al.,¹⁸ who investigated a set of monofunctional Pt(II) complexes with tridentate ligands in which the number and position of amine

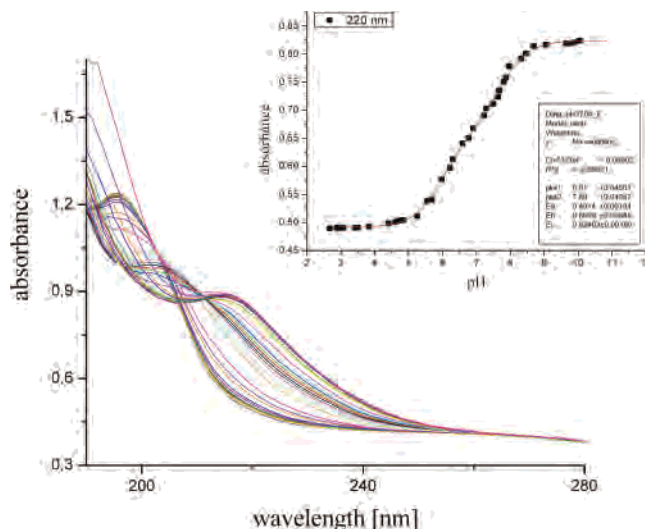


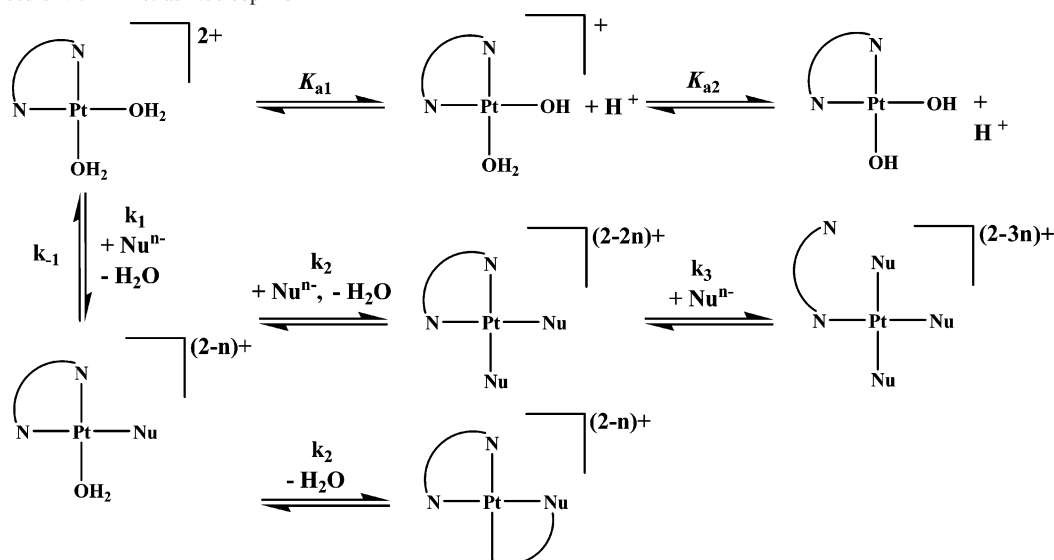
Figure 2. UV-vis spectra recorded for 0.015 mM Pt(dach) in the pH range of 2–10 at 25 °C. Inset: Plot of absorbance vs pH at 220 nm.

(a) and pyridine (p) groups were systematically varied, for example in **aaa**, **apa**, **aap**, and **ppp**. The following trend for the Pt–O bond lengths (B3LYP/LACVP**) was reported (Å): **aaa**, **aap** (2.149) < **apa** (2.153) < **app** (2.157) < **ppp** (2.163). This clearly indicates an increase in Pt–O bond length trans to pyridine, which increased further on increasing the number of pyridine groups in the chelate.

Deprotonation of Platinum-Bound Water. Figure 2 (see also Figures S1–S3, Supporting Information) shows an example of the spectral changes observed during the pH titration. In the pH range from 2 to 10 the spectra exhibit three different absorbance maxima, which shift toward longer wavelengths with increasing pH. This suggests that there are three species present in solution as a function of pH (viz.,

- (24) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, revision C.02; Gaussian, Inc.: Wallingford, CT, 2004.
- (25) Becke, A. D. *J. Phys. Chem.* **1993**, *97*, 5648–5652.
- (26) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785–789.
- (27) Stephens, P. J.; Devlin, F. J.; Chabalowski, C. F.; Frisch, M. J. *J. Phys. Chem.* **1994**, *98*, 11623–11627.
- (28) Dunning, T. H. J.; Hay, P. J. *Mod. Theor. Chem.* **1976**, 1–28.
- (29) Hay, P. J.; Wadt, W. R. *J. Chem. Phys.* **1985**, *82*, 270–310.
- (30) *Gaussian Basis Sets for Molecular Calculations*; Huzinaga, S. Ed.; Elsevier: Amsterdam, 1984.
- (31) The performance of this method (RB3LYP/LANL2DZp) is well documented (e. g., Saalfrank, R. W.; Deutscher, C.; Maid, H.; Ako, A. M.; Sperner, S.; Nakajima, T.; Bauer, W.; Hampel, F.; Hess, B. A.; van Eikema Hommes, N. J. R.; Puchta, R.; Heinemann, F. W. *Chem.–Eur. J.* **2004**, *10*, 1899–1905).
- (32) Illner, P.; Zahl, A.; Puchta, R.; van Eikema Hommes, N. J. R.; Wasserscheid, P.; van Eldik, R. *J. Organomet. Chem.* **2005**, *690*, 3567–3576.

Scheme 1. Summary of All Reactions Studied. Only the Kinetics of the Diaqua Species were Investigated (see Experimental Section). The Lowest Reaction only Occurs with L-Met as Nucleophile



the diaqua, aqua-hydroxo, and dihydroxo complexes). The overall process can therefore be presented by the reaction shown in the upper part of Scheme 1.

Plots of absorbance versus pH at specific wavelengths were used to determine the pK_a values of the coordinated water molecules. The data points were fitted using a nonlinear least-squares procedure, as represented by the insets in Figures 2 and S1–S3. The pK_a values obtained are summarized in Table 1.

The results clearly show a correlation between the pK_a values and the diamine chelate trans to the bound water molecules. The introduction of an increasing number of pyridine rings results in a decrease in electron density on the Pt(II) center.¹⁸ As a consequence, lower pK_a values can be observed for the second deprotonation step of **Pt(amp)** ($pK_{a2} = 6.83$) and in both steps of **Pt(bpy)** ($pK_{a1} = 4.80$, $pK_{a2} = 6.32$) as summarized in Table 1. The pK_a values found for **Pt(dach)** ($pK_{a1} = 6.01$, $pK_{a2} = 7.69$) and **Pt(en)** ($pK_{a1} = 5.97$, $pK_{a2} = 7.47$) are typical for sp^3 -hybridized nitrogen in amines.^{33–35} A comparison with values for the diaqua form of cisplatin ($pK_{a1} = 5.37$, $pK_{a2} = 7.21$) shows a decrease in the pK_a values with the decreasing σ donor ability of the ligands: $-\text{NH}_3 < \text{NH}_2\text{R} < \text{NHR}_2$ in the corresponding complexes, $\text{cisplatin}-(\text{OH}_2)_2 < \text{Pt(en)} < \text{Pt(dach)}$.

Since the **Pt(amp)** complex contains both types of nitrogens, the aromatic pyridine and the sp^3 -hybridized primary amine, it is interesting to know which water molecule is deprotonated first: the one trans to the pyridine or the one trans to the amine. Since the electron density of the hydroxo-ligand formed could be better stabilized by the pyridine than by the amine ligand, one might expect the first step to occur trans to the pyridine ring and therefore at lower pH than is the case for **Pt(dach)** and **Pt(en)**. But the first pK_{a1} value of

5.82 for **Pt(amp)** is on the same order of magnitude as that found for **Pt(dach)** (6.01) and **Pt(en)** (5.97). The computed (RB3LYP/LAN2DZp)^{24–30} energies of the cis and trans mono-hydroxo-**Pt(amp)** complexes corroborate this: the stability difference between the isomers (0.2 kcal/mol) is negligible (i.e., there is no preference for deprotonation trans to the pyridine ring).

The energy profile for proton exchange between the aqua and hydroxo ligand in **Pt(amp)** is shown in Figure 3. The calculated barrier is only 1.6 kcal/mol. Thus, proton transfer between the O-donor ligands will be essentially activation-less, and it is impossible to assign the first deprotonation to a specific site. Note that an intramolecular H bridge between two ligands, a prerequisite for intramolecular H migration, is not an unusual motif in late transition metal complex chemistry (e.g., in $\text{K}_3[\text{Pt}\{(\text{SO}_3)_2\text{H}\}\text{Cl}_2]$ ^{36,37} or *cis*-MH[P(O)Ph₂]-[PPh₂(OH)](PEt₃) (M = Pd, Pt)).³⁸ Analogously, fast proton exchange is also expected for the other complexes, but we did not pursue this as no difference in deprotonation would be observable experimentally because of the symmetry of the complexes.

The value of 6.83 for the second deprotonation step in **Pt(amp)** can be considered an average of the values of **Pt(en)** (7.47) and **Pt(bpy)** (6.32), which would be 6.89, as both structure motifs, pyridine and amine, are combined in the **Pt(amp)** complex. The incoming electron density from the first hydroxo ligand can be stabilized by the electron-withdrawing ability of the π -accepting pyridine ring, thus resulting in a decrease in pK_{a2} as compared to **Pt(dach)** and **Pt(en)**. **Pt(bpy)** of course exhibits the largest withdrawing-electron density and shows in both steps considerably low pK_a values.

(33) Coley, R. F.; Martin, D. S. *Inorg. Chim. Acta* **1973**, *7*, 573.

(34) Barton, S. J.; Barnham, K. J.; Habtemariam, A.; Sue, R. E.; Sadler, P. J. *Inorg. Chim. Acta* **1998**, *273*, 8–13.

(35) Berners-Price, S. J.; Frenkiel, T. A.; Frey, U.; Ranford, J. D.; Sadler, P. J. *J. Chem. Soc., Chem. Commun.* **1992**, 789.

(36) Howard, J.; Tomkinson, J.; Eckert, J.; Goldstone, J. A.; Taylor, A. D. *Chem. Phys.* **1983**, *78*, 3150–3155.

(37) Kehr, W. G.; Breiting, D. K.; Bauer, G. *Acta Crystallogr.* **1980**, *B36*, 2545–2550.

(38) Han, L.-B.; Choi, N.; Tanaka, M. *Organometallics* **1996**, *15*, 3259–3261.

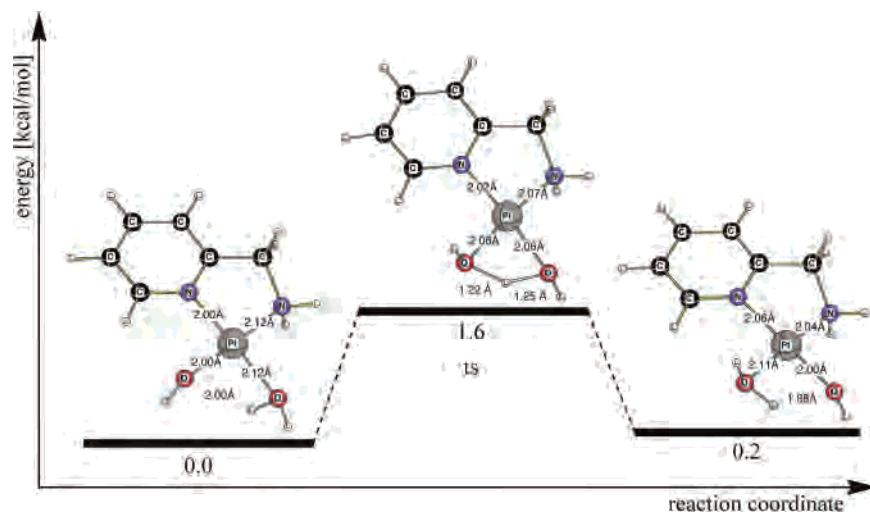


Figure 3. Calculated energy (B3LYP/LANL2DZp) profile for intramolecular proton transfer of the monodeprotonated Pt(amp) complex with transition state (ts).

It is well-known that pK_a values of coordinated water serve as an experimental indicator for the electron density around the metal center.³⁹ Especially in terms of the application of Pt(II) in cancer therapy, such investigations play an important role. Independent of the leaving group of the parent metal complex, for instance chloride in cisplatin or dicarboxylates in oxaliplatin (oxalate) and carboplatin (cyclobutyl-dicarboxylate),⁶ in all cases aquation processes in the cell nucleus convert the Pt(II) drugs to more reactive diaqua or aqua-hydroxo species that react with the final DNA target.⁴⁰ Therefore, it is essential to know which species are predominant at a physiological pH of 7.4. In this respect, the stabilizing effect of the pyridine rings leads to an increased concentration of the dihydroxo species which are practically inert to substitution reactions,^{41,42} most probably because of the back-bonding ability of the lone-pair electrons of the hydroxo ligand to the p_z orbital of the metal. The pK_a values of Pt(en) and Pt(dach) exhibit a more suitable species distribution at physiological pH. On the other hand, it is known that tumor cells prevalently degrade glucose anaerobically to lactic acid instead of aerobically to water and carbon dioxide,⁴³ resulting in pH values below 6.8.⁴⁴ Hence a Pt(II) drug which is more active in acidic medium than at physiological pH would exhibit beneficial properties with respect to its DNA binding ability. At pH 6.6, the Pt(amp) complex for instance would exist 65% in its active diaqua and aqua-hydroxo forms and only 35% in its inert dihydroxo form, whereas at pH 7.4, already 66% would be converted to its inert dihydroxo form. Thus, depending on the diamine moiety, the electrophilicity of the metal center can be tuned toward a more favorable reactivity under the given conditions.

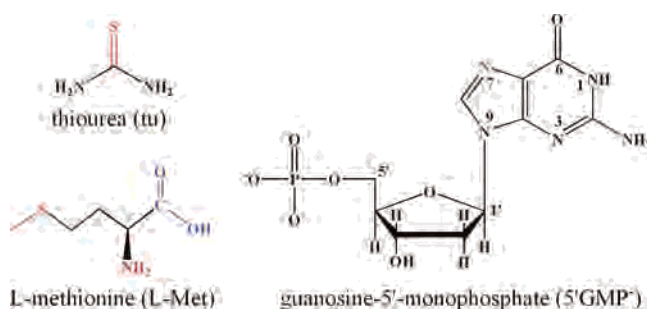


Figure 4. Structures of the investigated nucleophiles.

Kinetic Measurements. The substitution reactions were performed at pH 2.0–2.2 so that the activity could be determined when the complexes were predominantly in their diaqua form (see data in Table 1). Protonation of tu ($pK_a = -1.3$) under given conditions can be neglected, as recently reported.¹⁵ The kinetics of the substitution of coordinated water molecules (see Scheme 1) was investigated spectrophotometrically by following the change in absorbance at suitable wavelengths (Table S1) as a function of time using UV–vis and stopped-flow techniques. Thiourea (tu), L-methionine (L-Met), and guanosine-5'-monophosphate ($5'GMP^-$) were investigated as nucleophiles because of their different nucleophilicity, steric hindrance, binding properties, and biological relevance (see structures shown in Figure 4). As mentioned above, all kinetic experiments were performed under pseudo-first-order conditions with respect to either the Pt(II) complex or the nucleophile to force the reactions to go to completion.

Reactions with tu. The substitution of the aqua ligands by tu involves two reaction steps, with reaction rates differing only by a small factor between 2 and 6. The largest difference in rate constants between the first and second step was observed for the Pt(amp) complex, likely because of the different electronic nature and trans effect of the coordinated N donors. The pyridine ring is a π -acceptor with a strong Pt–pyridine bond, and it exhibits a larger trans effect than the NH_2R group. Because of the small rate difference, it is usually difficult to mathematically separate the two rate

(39) Dadci, L.; Elias, H.; Frey, U.; Hörmig, A.; Koelle, U.; Merbach, A. E.; Paulus, H.; Schneider, J. *S. Inorg. Chem.* **1995**, *34*, 306.

(40) Lippert, B. e. *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*; Wiley-VCH: Zürich, Switzerland, 1999; 183–221.

(41) Mahal, G.; van Eldik, R. *Inorg. Chem.* **1985**, *24*, 4165.

(42) Mahal, G.; van Eldik, R. *Inorg. Chim. Acta* **1987**, *127*, 203.

(43) Warburg, O. *Über den Stoffwechsel von Tumoren*; Springer: Berlin, 1926.

(44) Gerweck, L. E. *Semin. Radiat. Oncol.* **1998**, *8*, 176–182.

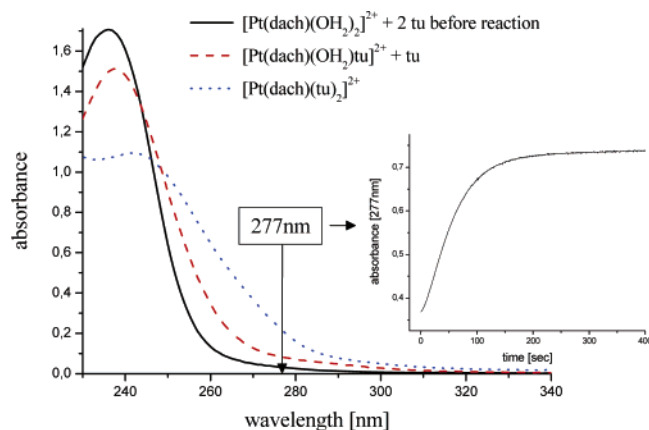


Figure 5. UV-vis spectra of the reactants, intermediates, and products for the two reaction steps. $[\text{Pt}(\text{dach})] = 0.1 \text{ mM}$, $\text{pH} = 2$, $I = 0.01 \text{ M}$, $T = 25 \text{ }^\circ\text{C}$. The spectrum for the monosubstituted complex was obtained from an equilibrated solution containing platinum in a 4-fold excess over thiourea and was corrected for the excess platinum background. The spectrum of an equimolar amount of uncoordinated tu was added mathematically to show the overall absorbance change observed for the formation of the 1:1 complex. Inset: Sigmoidal time trace at 277 nm.

constants. However, as described by Fekl et al.,⁴⁵ both reaction rates can be determined accurately when the first reaction step involves a much smaller absorbance change than the second step. The spectra, Figure 5 (see also Figure S4, Supporting Information), show that this is indeed the case. The absorbance change for the second substitution step at 277 nm is about 3 times larger than for the first step. As demonstrated in the inset in Figure 5 (see also Figure S5, Supporting Information), the time trace at a suitable wavelength shows a sigmoidal shape resulting from an induction period and can be fitted to a two-exponential model in which the amplitudes have opposite signs.

A linear dependence of the observed rate constants on the tu concentration was found as shown for the **Pt(amp)** complex in Figure 6. $k_{\text{obs}1}$ and $k_{\text{obs}2}$ can be expressed by equations 1 and 2. All of the reactions studied do not show an intercept; therefore, the reactions are irreversible, and k_{-1} and k_{-2} are zero. By reducing the positive inductive effect

$$k_{\text{obs}1} = k_1[\text{Nu}] + k_{-1} \approx k_1[\text{Nu}]$$

$$\text{Nu} = \text{L-Met, tu, } 5'\text{GMP}^- \quad (1)$$

$$k_{\text{obs}2} = k_2[\text{Nu}] + k_{-2} \approx k_2[\text{Nu}] \quad \text{Nu} = \text{tu, } 5'\text{GMP}^- \quad (2)$$

of the diaminocyclohexane ring in **Pt(dach)** on going to the **Pt(en)** complex, an enhancement of about 50% (**Pt(dach)**, $21.4 \text{ M}^{-1} \text{ s}^{-1}$; **Pt(en)**, $34.0 \text{ M}^{-1} \text{ s}^{-1}$) was observed for the first substitution step (k_1) in the reaction with tu. The introduction of one pyridine ring (**Pt(amp)**, $233 \text{ M}^{-1} \text{ s}^{-1}$) results in an additional acceleration of a factor of about 7, and the second π -acceptor group in the **Pt(bpy)** complex leads to a further enhancement factor of 22 ($5081 \text{ M}^{-1} \text{ s}^{-1}$). Studies on Pt(II) complexes with tridentate ligands with a different combination and number of pyridine and amine ligands¹⁸ showed a multiplicative effect on the enhancement of substitution reactions of one water molecule if the pyridine

(45) Fekl, U.; van Eldik, R. *Eur. J. Inorg. Chem.* **1998**, 389–396.

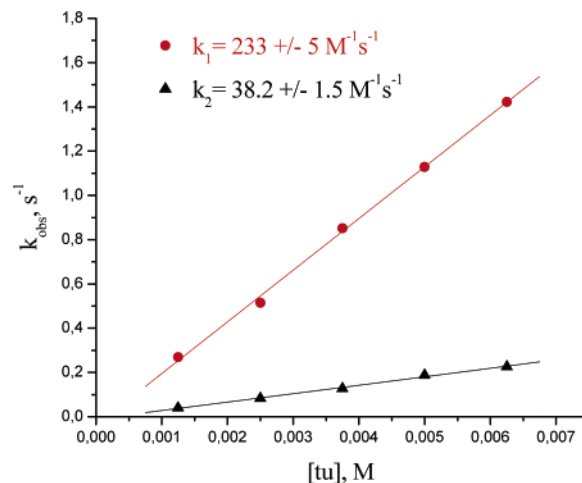


Figure 6. Plots of k_{obs} vs tu concentration for the **Pt(amp)** complex (0.125 mM). $I = 0.01 \text{ M}$ (HOTf), $T = 25 \text{ }^\circ\text{C}$.

rings are spatially separated as, for example, in $[\text{Pt}(\text{bis}(2\text{-pyridylmethyl)amine})\text{OH}_2]\text{ClO}_4$. In the case where pyridine rings are adjacent to each other as in $[\text{Pt}(\text{bpy})(\text{NH}_3)(\text{OH}_2)]\text{ClO}_4$, the platinum center was found to be more electrophilic and therefore more reactive by a much larger increase in the rate constant than just a multiplicative effect. This finding was attributed to the effect of “electronic communication” between the chelate subunits that results in very efficient π -back-bonding.⁴⁶ This also matches the results of the present work. A multiplicative effect would result in an acceleration factor of about 49 on going from **Pt(en)** to **Pt(bpy)**, but our results show an overall factor of 150.

The reaction with tu shows a second slower substitution step, which can be attributed to steric hindrance of coordinated thiourea and to its electron donation toward the Pt(II) center which makes it less electrophilic. No difference between **Pt(en)** ($10.2 \text{ M}^{-1} \text{ s}^{-1}$) and **Pt(dach)** ($11.5 \text{ M}^{-1} \text{ s}^{-1}$) could be detected for k_2 . Only about half of the accelerating effect of k_1 , namely, 3.8, was observed on introduction of one pyridine ring (**Pt(amp)**, $38.2 \text{ M}^{-1} \text{ s}^{-1}$). Again, a high enhancement factor of 110 was found for the reaction with **Pt(bpy)** ($1119 \text{ M}^{-1} \text{ s}^{-1}$), compared to **Pt(en)/Pt(dach)**.

For the substitution reaction with tu, a third concentration dependent step was observed that can be attributed to substitution of the now labilized amine trans to tu, as a result of the strong trans effect of tu. Comparable reactions are known, for instance, in the biotransformation pathway of cisplatin, in which the amines are substituted by sulfur-containing nucleophiles.^{47,48} This is of great importance, as the resulting reaction products (e.g., $[\text{Pt}(\text{L-Met-S,N})_2]$) are inert to further substitution reactions and therefore limit the active concentration of the drug. The diamine chelates used in this study are more stable toward substitution than amines in cisplatin or carboplatin. The subsequent third step was studied only for **Pt(dach)** (see Figure 7) and **Pt(en)** with tu,

(46) Jaganyi, D.; Hofmann, A.; van Eldik, R. *Angew. Chem., Int. Ed.* **2001**, *40*, 1680.

(47) Appleton, T. G.; Connor, J. W.; Hall, J. R. *Inorg. Chem.* **1988**, *27*, 130–137.

(48) Norman, R. E.; Sadler, P. J. *Inorg. Chem.* **1988**, *27*, 3583–3587.

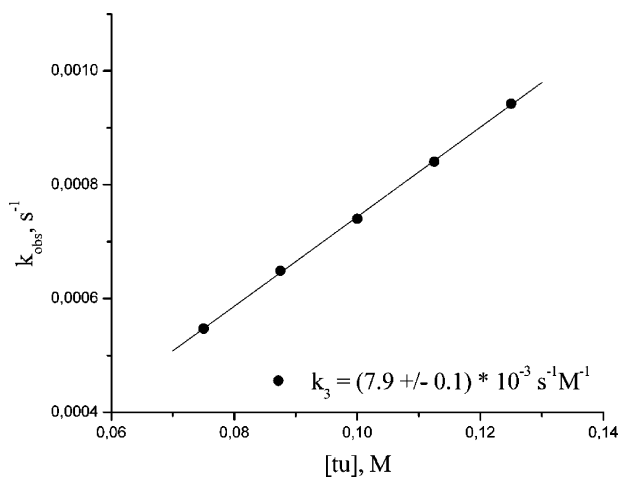


Figure 7. Plots of k_{obs} vs tu concentration for the **Pt(dach)** complex (0.125 mM). $I = 0.01$ M (HOTf), $T = 25$ °C.

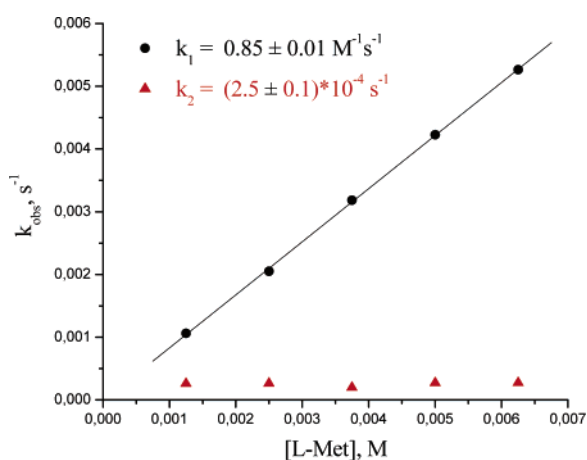


Figure 8. Plots of k_{obs} versus L-Met concentration for the **Pt(dach)** complex (0.125 mM). $I = 0.01$ M (HOTf), $T = 25$ °C, $\lambda = 240$ nm.

which has a stronger trans effect than L-Met, and in the presence of a very large excess of tu because of the long duration of the reaction. The observed rate constants for k_3 are summarized in Table 3 and demonstrate that the more flexible ethylenediamine moiety can be substituted faster ($22.3 \text{ M}^{-1} \text{ s}^{-1}$) than the sterically more demanding *trans*-1,2-diaminocyclohexane ligand ($7.9 \text{ M}^{-1} \text{ s}^{-1}$).

Reactions with L-Met. Kinetic traces for reactions with L-Met gave excellent fits to a double exponential function (see Figures S7 and S12; UV/vis spectral changes, Figures S6, S10, and S11, Supporting Information). The so-obtained constants, $k_{\text{obs}1}$ and $k_{\text{obs}2}$, were plotted against the concentration of the entering L-Met molecule. In the case of $k_{\text{obs}1}$, a linear dependence on the nucleophile concentration was observed for all the complexes studied; $k_{\text{obs}2}$ was found to be independent of the L-Met concentration, suggesting a chelate formation process. Plots showing representative results for one complex are shown in Figure 8. The results for the reactions with L-Met imply that $k_{\text{obs}1}$ and $k_{\text{obs}2}$ can be expressed by eqs 1 and 3.

$$k_{\text{obs}2} = k_2 \quad \text{Nu} = \text{L-Met} \quad (3)$$

The nucleophilic attack occurs via the sulfur donor of the thioether group, and subsequently a six-membered ring (see

Scheme 2. Second Substitution Step in the Reaction with L-Met Involving Deprotonation

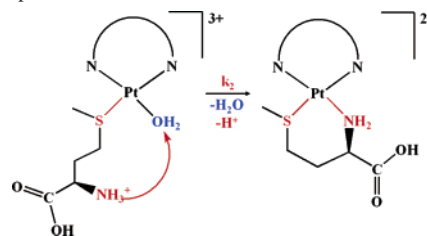


Table 2. Summary of the Second-Order Rate Constants for the Displacement of the First Coordinated Water Molecule by a Range of Nucleophiles in Complexes of the Type $[\text{Pt}^{\text{II}}(\text{N}-\text{N})(\text{OH}_2)_2]^{2+}$

	T (°C)	pH	k_1 ($\text{M}^{-1} \text{s}^{-1}$)			
			Pt(dach)	Pt(en)	Pt(amp)	Pt(bpy)
tu	25	2	21 ± 1	34.0 ± 0.4	233 ± 5	5081 ± 275
L-Met	25	2	0.85 ± 0.01	0.70 ± 0.03	2.15 ± 0.05	21.8 ± 0.6
	25	2		0.71 ± 0.02^a		
	40	2			4.1 ± 0.1	
5'GMP	40	4			4.14 ± 0.05	
	40	2	5.8 ± 0.2	3.9 ± 0.1	12.5 ± 0.5	24.4 ± 0.3

^a Kinetics measured with complex in excess.

Scheme 2) is formed by the nitrogen donor of the amine group. To further confirm this chelation step, the kinetics were also studied with complex **Pt(en)** in excess instead of L-Met, so that a two step reaction can only occur if ring-closure is involved and substitution of the second water molecule by L-Met can be excluded. As expected, the kinetic traces could be fitted perfectly to a double exponential model, and gave the same values for the rate constants within the experimental error limits as in the experiment with L-Met in excess.

In light of the acid dissociation constants of free L-Met ($\text{p}K_{\text{COOH}} = 2.28$,⁴⁹ 2.13 ;⁵⁰ $\text{p}K_{\text{NH}_3^+} = 9.2$), ring-closure involves deprotonation of the amine group of the amino acid. In addition, the $\text{p}K_{\text{a}}$ values of L-Met decrease significantly upon metal ion coordination. Therefore, at $\text{pH} > 2$, deprotonation has to be more efficient because of the lower proton concentration in solution. The reaction was therefore repeated at pH 4 (see Figure S9, Supporting Information) with **Pt(amp)** (where the complex is still in the diaqua form). The first step was found to be independent of the proton concentration, as expected, since substitution of a water molecule by the thioether group would not be affected by pH. But the second step was accelerated by a factor of 1.7 on decreasing the proton concentration by a factor of 100 as can be seen in Table 2. Thus, the amine protons are released more easily in solutions with lower proton concentration allowing the S,N-chelate ring to form. This step is irreversible. Model DFT calculations (RB3LYP/LAN2DZp)^{24–32} show that $[\text{Pt}(\text{NH}_3)_2(\text{S}-\text{N})]^{2+}$ is 6.68 kcal/mol more stable than the corresponding S,O-isomer at pH 2 (L-Met⁺). No significant difference (0.32 kcal/mol) between S,O/S,N chelation was found for the zwitterionic L-Met molecule.

(49) Cohn, E. J.; Edsall, J. T. *Proteins, Amino Acids and Peptides as Ions and Dipolar Ions*; Reinhold Publishing Corporation: New York, 1943.

(50) Lide, D. R. *Handbook of Chemistry and Physics*; CRC Press: New York, 1995.

Table 3. Summary of the Rate Constants for the Displacement of the Second Coordinated Water Molecule by a Range of Nucleophiles in Complexes of the Type $[\text{Pt}^{\text{II}}(\text{N}-\text{N})(\text{OH}_2)_2]^{2+}$ and Rate Constant for the Third Reaction Step with Tu

	T (°C)	pH		Pt(dach)	Pt(en)	Pt(amp)	Pt(bpy)
tu	25	2	k_2 ($\text{M}^{-1} \text{s}^{-1}$)	11.5 ± 0.5	10.2 ± 0.2	38 ± 1	1119 ± 22
tu	25	2	$k_3 \times 10^3$ ($\text{M}^{-1} \text{s}^{-1}$)	7.9 ± 0.1	22.3 ± 0.1		
L-Met	25	2	$k_2 \times 10^4$ ($\text{M}^{-1} \text{s}^{-1}$)	2.5 ± 0.1	2.0 ± 0.2	1.2 ± 0.3	290 ± 4
L-Met ^a	25	2			1.9 ± 0.1		
L-Met ^b	40	2				2.8 ± 0.2	
L-Met ^c	40	4				4.8 ± 0.7	
5'GMP	40	2	k_2 ($\text{M}^{-1} \text{s}^{-1}$)	0.21 ± 0.02	0.38 ± 0.02	0.97 ± 0.02	24 ± 1

^a Kinetics measured with complex in excess. ^b For concentration dependence, see Figure S8 (Supporting Information) ^c For concentration dependence, see Figure S9 (Supporting Information).

NMR experiments by Appleton et al.⁴⁷ also confirm the S,N-chelate to be the only significant species present in solution. The initial formation of a thermodynamically less favored seven-membered ring via the oxygen of the carboxylic acid group that subsequently isomerizes to the S,N-chelate, only occurs in very acidic media (pH < 0.5) as this strongly kinetically inhibits S,N-chelate formation.⁴⁷

The k_1 values for the reaction with L-Met increase by a factor of 3 on going from **Pt(dach)** ($0.85 \text{ M}^{-1} \text{ s}^{-1}$)/**Pt(en)** ($0.70 \text{ M}^{-1} \text{ s}^{-1}$) to **Pt(amp)** ($2.15 \text{ M}^{-1} \text{ s}^{-1}$), with a further enhancement by a factor of 10 on the addition of a second pyridine ring in **Pt(bpy)** ($21.8 \text{ M}^{-1} \text{ s}^{-1}$). As has already been pointed out, the electronic communication⁴⁶ in the **Pt(bpy)** system results in a more electrophilic metal center and an increase in the reaction rate that can not only be described by a multiplicative effect, as in the case of spatially separated pyridine rings.¹⁸ The enhanced reactivity can be explained by π -back-donation of the incoming electron density from the nucleophile to the pyridine chelate. This stabilizes the five-coordinate transition state relative to the ground state, since the ground state cannot benefit much from the π -back-donation given that the $6p_z$ orbital of the Pt center is empty.⁵¹ In the chelation step, only the k_2 value of the **Pt(bpy)** ($290 \text{ M}^{-1} \text{ s}^{-1}$) complex is significantly higher than that for **Pt(dach)** ($2.50 \text{ M}^{-1} \text{ s}^{-1}$), **Pt(en)** ($2.04 \text{ M}^{-1} \text{ s}^{-1}$), and **Pt(amp)** ($1.2 \text{ M}^{-1} \text{ s}^{-1}$). As the k_{obs} values for the first and second step of **Pt(bpy)** are very similar, it was not possible to clearly separate them. Because of this, only the rate constants were investigated for both steps.

Reactions with 5'GMP⁻. It is known that the N7 site of 5'GMP⁻ is strongly favored for binding to metal ions,⁴⁰ but in principle, both N1 and N7 of 5'GMP⁻ (see Figure 4) can coordinate, depending on the pH of the solution. N1 protonation ($\text{p}K_{\text{a}}(\text{N1}_{\text{free}}) = 9.3$, $\text{p}K_{\text{a}}(\text{N1}_{\text{coord}}) = 3.7$)⁵² reduces the availability of this site in neutral and acidic solution, and this position is also sterically hindered by the amino group at C2.⁴⁰ Therefore, in this study a pH of 2.0–2.2 was selected to ensure that only N7 ($\text{p}K_{\text{a}}(\text{N7}_{\text{free}}) = 2.48$) will coordinate. 65 to 75% of the 5'GMP⁻ is protonated at N7, and therefore the molecule has no charge; 25–35% of the 5'GMP⁻ has a negative charge because of a single deprotonated phosphate group. N7 of the negatively charged 5'GMP⁻ reacts with

the Pt(II) center, and the acid–base equilibrium rapidly forms nonprotonated N7.



The reactions with 5'GMP⁻ revealed two substitution steps to give $[\text{Pt}(\text{N}-\text{N})(\text{N7-GMP})_2]$ as the final product. Thus $k_{\text{obs}1}$ and $k_{\text{obs}2}$ can be expressed by eqs 1 and 2 and plots of k_{obs} versus excess complex (k_1) or nucleophile (k_2) result in a linear concentration dependence. Because of the high absorbance of 5'GMP⁻ in the UV–vis range, the first reaction step could not be observed with 5'GMP⁻ in excess and was therefore studied with complex in excess. The resulting kinetic traces gave excellent fits to a single-exponential function for the first step (see Figure S13, Supporting Information). The values of k_1 for the reaction with 5'GMP⁻ increase on going from **Pt(en)** ($3.9 \text{ M}^{-1} \text{ s}^{-1}$) and **Pt(dach)** ($5.8 \text{ M}^{-1} \text{ s}^{-1}$) to **Pt(amp)** ($12.5 \text{ M}^{-1} \text{ s}^{-1}$). The addition of a second pyridine ring in **Pt(bpy)** ($24.4 \text{ M}^{-1} \text{ s}^{-1}$) only accelerates the reaction by another factor of 2. The substitution of a second water molecule by 5'GMP⁻ occurs more than 10 times slower for **Pt(en)** ($0.38 \text{ M}^{-1} \text{ s}^{-1}$), **Pt(dach)** ($0.21 \text{ M}^{-1} \text{ s}^{-1}$), and **Pt(amp)** ($0.97 \text{ M}^{-1} \text{ s}^{-1}$). The first 5'GMP⁻ molecule occupies space and sterically hinders the second molecule to enter the coordination sphere. A clear separation of the first and second substitution steps in **Pt(bpy)** could not be observed. The experiment with the **Pt(bpy)** complex in excess gave a value ($24.4 \text{ M}^{-1} \text{ s}^{-1}$) on the same order of magnitude as that of 5'GMP⁻ ($24 \text{ M}^{-1} \text{ s}^{-1}$) in excess. From this observation, we can only conclude that the second step of the highly reactive **Pt(bpy)** complex apparently occurs on the same time scale as the first step.

Figure 9 shows the ¹H NMR spectrum of $[\text{Pt}(\text{dach})(\text{N7-GMP})_2]$ in comparison to that of free 5'GMP⁻ to confirm the nature of the reaction product. The region of the H1' and H8 protons is most suitable to reflect the changes from coordination at N7. As the *trans*-1,2-diaminocyclohexane–Pt(II) complex was used, a mixture of the S,S and R,R isomers can be seen in terms of two signals for H8 ($\delta = 8.66, 8.59$ ppm) and two doublets for H1' ($\delta = 5.96, 5.94$, $^3J_{\text{HH}} = 6.1$ Hz; $5.93, 5.91$, $^3J_{\text{HH}} = 6.6$ Hz). Since N1 and N7 are protonated in strongly acidic solution, electron density is withdrawn from H8, resulting in a more downfield position in comparison to the shifts obtained at higher pH.⁵² The signals for the H1' and H8 proton of free 5'GMP⁻ are

(51) Otto, S.; Elding, L. I. *J. Chem. Soc., Dalton Trans.* **2002**, 11, 2354–2360.

(52) Zhu, S.; Matilla, A.; Tercero, J. M.; Vijayaragavan, V.; Walmsley, J. A. *Inorg. Chim. Acta* **2004**, 357, 411–420.

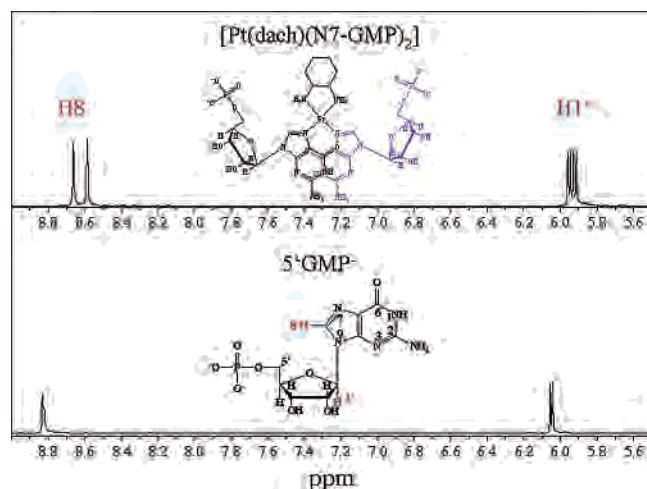


Figure 9. ^1H NMR spectra of $5'\text{GMP}^-$ and $[\text{Pt}(\text{dach})(\text{N7-GMP})_2]$ (10mM) at $\text{pH}^* = 2$ (DOTf), $T = 25^\circ\text{C}$.

shifted toward higher field following coordination to the Pt(II) center at pH 2.

All Nucleophiles. The reactivity order for the Pt(II) aqua complexes (viz., $\text{Pt}(\text{dach}) \approx \text{Pt}(\text{en}) < \text{Pt}(\text{amp}) \ll \text{Pt}(\text{bpy})$) confirms what was already expected on the basis of the $\text{p}K_a$ values (viz., the obvious influence of the diamine spectator ligands on the reactivity of the Pt(II) center). Addition of π -acceptors to the complex results in an increased reactivity of the Pt(II) center.

The reactions with the $\text{Pt}(\text{dach})$ complex were expected to be slower than those with $\text{Pt}(\text{en})$, as the Pt(II) center should be less electrophilic because of the positive inductive effect of the cyclohexane ring. In this respect, a clear trend could not be observed in the reactions studied. In terms of the stability of the complex, the *trans*-1,2-diaminocyclohexane moiety in $\text{Pt}(\text{dach})$ is more stable toward substitution reactions than the ethylenediamine in $\text{Pt}(\text{en})$. The ethylenediamine chelate is substituted by a factor of 2.8 faster (k_3 for the substitution by tu) than the *trans*-1,2-diaminocyclohexane moiety.

From a comparison of the reactivity of tu, L-Met, and $5'\text{GMP}^-$ in the reaction with the different Pt(II) complexes, it can be concluded that with increasing electrophilicity the reactivity is increased by an enhanced nucleophilic discrimination of the complex. While tu is clearly the strongest nucleophile, the N-donor nucleophile $5'\text{GMP}^-$ exhibits an affinity for Pt(II) complexes which is as good as the S-donor L-Met under these conditions. Although the HSAB theory⁵³ favors a stronger interaction of Pt ions with S-donor ligands, it seems that the conditions under which the reactions proceed are very important for the kinetic competition of N- and S-donor ligands. At pH 2–2.2, $5'\text{GMP}^-$ will react as a monoanionic species, while L-Met ($\text{p}K_a = 2.13$,^{50,2.28}⁴⁹) is up to 42% cationic and 58% neutral. In this case $5'\text{GMP}^-$ reacts faster than L-Met.

Activation Parameters. The activation parameters were determined through a systematic variation of temperature and pressure. The thermal activation parameters ΔH^\ddagger and ΔS^\ddagger

Table 4. Activation Parameters for the Reactions of Different Nucleophiles with Complexes of the Type $[\text{Pt}^{\text{II}}(\text{N-N})(\text{OH}_2)_2]^{2+}$

complex	nucleophile	ΔH_1^\ddagger (kJ mol ⁻¹)	ΔS_1^\ddagger (J K ⁻¹ mol ⁻¹)	ΔV_1^\ddagger (cm ³ mol ⁻¹)
Pt(dach)	tu	58 ± 3	-22 ± 9	
	L-Met	51 ± 2	-76 ± 5	-17 ± 2
	5'GMP	70 ± 1	-7 ± 3	
Pt(en)	tu	57 ± 3	-24 ± 11	
	L-Met	52 ± 2	-82 ± 7	-14 ± 1
	5'GMP	63 ± 3	-32 ± 11	
Pt(amp)	tu	52 ± 3	-28 ± 9	-6.27 ± 0.03
	L-Met	52 ± 2	-72 ± 6	-12 ± 1
	5'GMP	54 ± 2	-52 ± 8	
Pt(bpy)	tu	45 ± 2	-24 ± 7	
	L-Met	54 ± 1	-39 ± 3	-11.7 ± 0.2
	5'GMP	48 ± 4	-62 ± 12	

complex	nucleophile	ΔH_2^\ddagger (kJ mol ⁻¹)	ΔS_2^\ddagger (J K ⁻¹ mol ⁻¹)	ΔV_2^\ddagger (cm ³ mol ⁻¹)
Pt(dach)	tu	60 ± 2	-23 ± 6	
	L-Met	54 ± 6	-137 ± 20	-21 ± 3
	5'GMP	65 ± 1	-26 ± 3	
Pt(en)	tu	36 ± 2	-69 ± 6	
	L-Met	52 ± 2	-92 ± 4	-24 ± 4
	Pt(amp)	tu	35 ± 1	-95 ± 3
Pt(bpy)	L-Met	41 ± 5	-181 ± 16	
	tu	34 ± 2	-73 ± 5	

were calculated using the Eyring equation, and the data is summarized in Table 4 together with the volumes of activation, ΔV^\ddagger , calculated from the slope of $\ln k$ versus pressure.

The trend in the reactivity of the complexes can also be seen in the activation enthalpies for the reactions involving the nucleophile tu. The higher the electrophilicity of the metal center, the smaller the activation enthalpy because of stabilization of the transition state (viz., $\Delta H_1^\ddagger(\text{Pt}(\text{dach})) = 58 \text{ kJ mol}^{-1}$, $\Delta H_1^\ddagger(\text{Pt}(\text{en})) = 57 \text{ kJ mol}^{-1}$, $\Delta H_1^\ddagger(\text{Pt}(\text{amp})) = 52 \text{ kJ mol}^{-1}$, $\Delta H_1^\ddagger(\text{Pt}(\text{bpy})) = 45 \text{ kJ mol}^{-1}$). This can also be seen in Figure 10 and Table 4. The same behavior can be observed for the values of ΔH_1^\ddagger for the reaction with $5'\text{GMP}^-$: $\Delta H_1^\ddagger(\text{Pt}(\text{dach})) = 70 \text{ kJ mol}^{-1}$, $\Delta H_1^\ddagger(\text{Pt}(\text{en})) = 63 \text{ kJ mol}^{-1}$, $\Delta H_1^\ddagger(\text{Pt}(\text{amp})) = 54 \text{ kJ mol}^{-1}$, and $\Delta H_1^\ddagger(\text{Pt}(\text{bpy})) = 48 \text{ kJ mol}^{-1}$. The ΔS^\ddagger values also become more negative for the reaction with $5'\text{GMP}^-$: $\Delta S_1^\ddagger(\text{Pt}(\text{dach})) = -7 \text{ J K}^{-1} \text{ mol}^{-1}$, $\Delta S_1^\ddagger(\text{Pt}(\text{en})) = -32 \text{ J K}^{-1} \text{ mol}^{-1}$, $\Delta S_1^\ddagger(\text{Pt}(\text{amp})) = -52 \text{ J K}^{-1} \text{ mol}^{-1}$, and $\Delta S_1^\ddagger(\text{Pt}(\text{bpy})) = -62 \text{ J K}^{-1} \text{ mol}^{-1}$. This can be interpreted in terms of a stronger associative character of the transition state due to a more electrophilic Pt(II) center.

The k_{obs} values for all reactions increased with increasing pressure and show a linear dependence as demonstrated in Figures 12 and 13 (see also Figure S14, Supporting Information). The acceleration of the reactions by pressure indicates an activation process that is consistent with an associative substitution mechanism and is dominated by bond making.⁵⁴ This is also supported by the negative ΔS^\ddagger values calculated from the temperature dependence of the reactions (Table 4).

The values for the activation entropy, ΔS^\ddagger , and the activation volume, ΔV^\ddagger , are significantly negative for all reactions studied. The second substitution step in the reaction

(53) Huheey, J.; Keiter, E.; Keiter, R. *Anorganische Chemie: Prinzipien von Struktur und Reaktivität*; Auflage: Berlin, 1995; Vol. 2.

(54) Tobe, M. L.; Burgess, J. *Inorganic Reaction Mechanisms*; Addison-Wesley Longman Inc.: Essex, U.K., 1999; p 70.

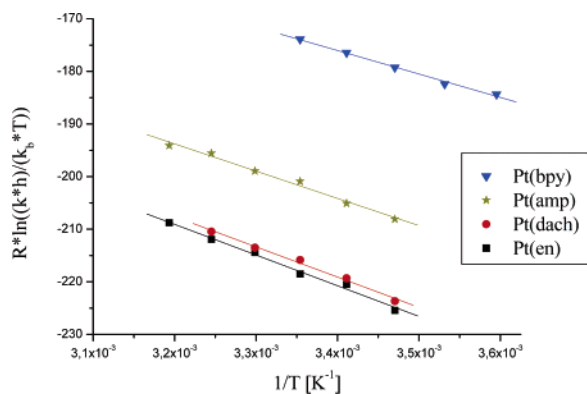


Figure 10. Eyring plots for the determination of the activation enthalpies and entropies for k_1 of all complexes studied with tu.

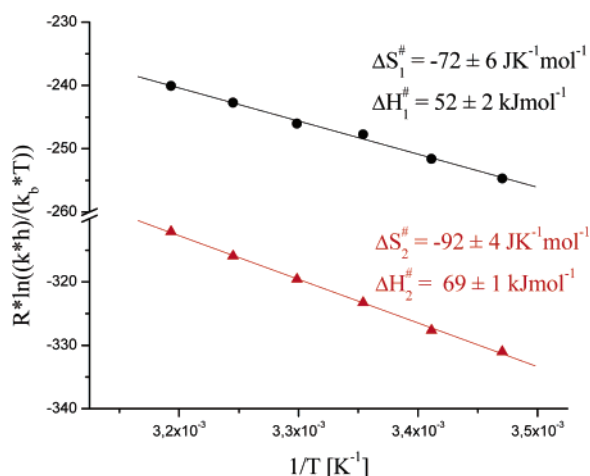


Figure 11. Eyring plots for the determination of the activation enthalpies and entropies for the two reaction steps of the **Pt(en)** complex with L-Met.

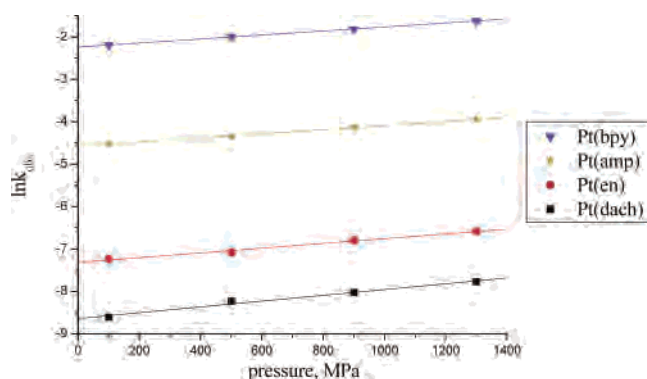


Figure 12. Plots of $\ln k_{\text{obs}}$ vs pressure for the first reaction step of all complexes studied with L-Met.

with L-Met, which involves chelate formation, shows a more negative activation entropy and activation volume compared to the first step. The highly negative ΔV^\ddagger value of $-24 \text{ cm}^3 \text{ mol}^{-1}$ for the second step of the reaction of L-Met with **Pt(en)**, for instance, can be assigned to chelate formation and the liberation of a proton to form $[\text{H}(\text{H}_2\text{O})_4]^+$. Values for the partial molar volume of a proton, $V_{\text{H}^+}^0$, were estimated by various methods in the past and reported by Millero^{55,56}

(55) Millero, F. J. *Water and Aqueous Solutions: Structure, Thermodynamics and Transport Properties*; Wiley-Interscience: New York, 1972; Chapter 13, p 532.

(56) Millero, F. J. *Chem. Rev.* **1971**, *71* (2), 163–169.

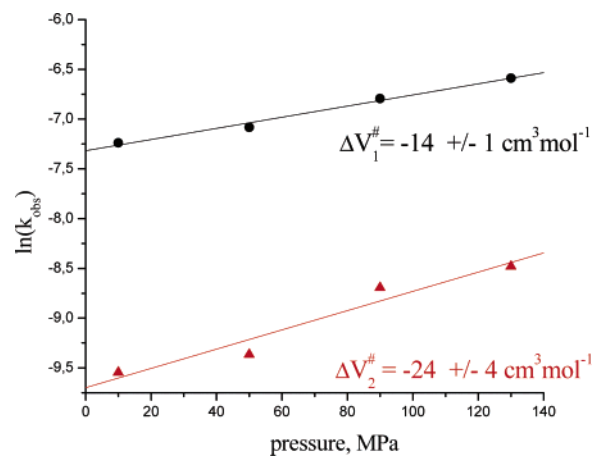


Figure 13. Plots of $\ln k_{\text{obs}}$ vs pressure for the reaction of the **Pt(en)** complex with L-Met.

to be $-4.2 \pm 1.5 \text{ cm}^3 \text{ mol}^{-1}$. Recently Sarauli et al.⁵⁷ calculated $V_{\text{H}^+}^0$ on the basis of a combination of the values for the reaction volumes from 19 previously performed neutralization reactions⁵⁸ with the reaction volume for the dissociation of a water molecule ($\Delta V^0 = -22.2 \pm 0.2 \text{ cm}^3 \text{ mol}^{-1}$ ^{59, 60}), which gave a value for $V_{\text{H}^+}^0$ of $-7.7 \pm 0.8 \text{ cm}^3 \text{ mol}^{-1}$. Thus a value of $V_{\text{H}^+}^0$ in the range of -4.2 to $-7.7 \text{ cm}^3 \text{ mol}^{-1}$ can be formally subtracted from the volume of activation of $-24 \text{ cm}^3 \text{ mol}^{-1}$ found for the second step of the reaction of **Pt(en)** with L-Met. The resulting value of -16.3 (-7.7) or $-19.8 \text{ cm}^3 \text{ mol}^{-1}$ ($-4.2 \text{ cm}^3 \text{ mol}^{-1}$) represents ΔV^\ddagger for the associative ring-closure reaction of coordinated L-Met.

The effect of high pressure could not be studied for all substitution reactions because of spectral limitations caused by the pressure medium employed (viz., *n*-heptane).

Conclusions

This study demonstrated that π -acceptors increase the electrophilicity on the Pt(II) metal center, due to their electron-withdrawing properties, which results in increasing reaction rates for nucleophilic substitution reactions. Especially when two pyridine rings are adjacent to each other as in **Pt(bpy)**, the rates increase significantly (compared to **Pt(dach)**/**Pt(en)**) by a factor of about 30–150, compared to a factor of 3–7 in the case of only one π -acceptor (**Pt(amp)**). This can be attributed to the stronger trans effect of π -acceptors such as pyridine rings compared to the weak trans effect of amines of the type NH_2R , NHR_2 . These findings are also reflected in the pK_a values of the coordinated water molecules, since they decrease with an increasing number of pyridine rings. Thus, hydroxo species can be stabilized more efficiently by pyridine-containing chelates than by RNH_2 chelates. Therefore, the pH can control the reactivity of the complex over the fraction of the aqua complex available in solution, which is important, considering that the hydrolysis product is the active species in the

(57) Sarauli, D.; Meier, R.; Liu, G.-F.; Ivanovic-Burmacovic, I.; van Eldik, R. *Inorg. Chem.* **2005**, *44*, 7624–7633.

(58) Kitamura, Y.; van Eldik, R. *Ber. Bunsen-Ges. Phys. Chem.* **1984**, *88*, 418–422.

mechanism of action of Pt(II) antitumor drugs. The computed energies for *cis*- and *trans*-monohydroxo-**Pt(amp)** show a low barrier (1.6 kcal/mol) between the two species and no stability difference between the isomers (0.2 kcal/mol). Thus, proton transfer between the O-donor ligands will essentially be activationless, and it is impossible to assign deprotonation to a specific site.

The acceleration of the reactions by pressure point to an activation process that is consistent with an associative substitution mechanism, which is also supported by the negative ΔS^\ddagger values calculated from the temperature dependence of the reactions.

In these studies, tu was found to be the strongest nucleophile, which, after substitution of two water molecules, labilizes the Pt–N bond in the *trans* position to form a ring-opened trisubstituted $[\text{Pt}(\text{tu})_3(\text{N}-\text{N}_{\text{open}})]^{2+}$ species.

The reaction with L-methionine proceeds first via substitution of a water molecule by sulfur, followed by a ring-closure step via nitrogen, and thus is independent of the nucleophile concentration. The second step depends on pH and exhibits very large negative values for ΔS^\ddagger and ΔV^\ddagger . This is attributed to the deprotonation of the amine during chelate formation. Such biotransformation products were also found in the urine of patients treated with Pt(II) cytostatics.⁶¹ It is therefore important to study the reactions of medically relevant Pt(II) complexes with L-Met since the reaction products are known to be involved in nephrotoxic side effects.

The DNA constituent 5'GMP[−] exhibits a high affinity for the selected Pt(II) complexes, which even exceeds the

reactivity of the Pt(II) diaqua complexes with L-Met. Thus, we demonstrated that a nitrogen donor can indeed compete for the Pt(II) center, depending on the conditions under which the reactions proceed.

The results have shown how the reactivity and $\text{p}K_{\text{a}}$ values of Pt(II) diaqua complexes can be electronically tuned by modifying the properties of the spectator ligands. The possibility of adjusting the properties of the drug to specific biological conditions may prove to be of importance in Pt(II) drug design.

Acknowledgment. The authors gratefully acknowledge stimulating discussions with Prof. Živadin Bugarčić and Prof. Ulrich Fekl, and financial support from the Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie. Prof. Tim Clark, Computer Chemistry Center, is kindly acknowledged for his support of this work. We thank the Regionales Rechenzentrum Erlangen (RRZE) for a generous allotment of computer time.

Supporting Information Available: Tables S1–S13 summarize all values for k_{obs} determined for all reactions at different concentrations and temperatures, Figures S1–S3 show the UV–vis spectra for the **Pt(amp)**, **Pt(en)**, and **Pt(bpy)** complexes in the pH range of 2–10 and plots of absorbance versus pH, Figures S4–S13 show different UV–vis spectral changes, kinetic traces, and concentration dependences, Figure S14 reports the pressure dependence for the second step of the reaction of **Pt(dach)** with L-Met, and Figure S15 summarizes the temperature dependence of k_1 for the reactions with 5'GMP[−]. This material is available free of charge via the Internet at <http://pubs.acs.org>.

(59) Millero, F. J.; Hoff, E. V.; Cahn, L. *J. Sol. Chem.* **1972**, *1*, 309.

(60) Kitamura, Y.; Itoh, T. *J. Sol. Chem.* **1987**, *16*, 715.

(61) Riley, C. M.; Sternson, L. A.; Repta, A. J.; Slyter, S. A. *Anal. Biochem.* **1983**, *130*, 203.

IC051955R